atgattcaaaaacgaaagcggacagtttcgttcagacttgtgcttatgtgcacgctgttatttgtcagttt gccgattacaaaaacatcagccGTAAATGGCACGCTGATGCAGTATT-TTGAATGGTATACGCCGAACGACGGCCAGCATT GGAAACGATTGCAGAATGATGCGGAA-CATTTATCGGATATCGGAATCACTGCCGTCTGGATTCCTCCCGCATACAAGGA TTGAG-5 CCAATCCGATAACGGATACGGACCTTATGATTTGTATGATTTAGGAGAATTCCAGCAAAA~ AGGGACGGTCAGAAC GAAATACGGCACAAAATCAGAGCTTCAAGATGCGATCGGCTCAC-TGCATTCCCGGAACGTCCAAGTATACGGAGATGTGG TTTTGAATCATAAGGCTGGTGCT-GATGCAACAGAAGATGTAACTGCCGTCGAAGTCAATCCGGCCAATAGAAATCAGGAA ACTTCGGAGGAATATCAAATCAAAGCGTGGACGGATTTTCGTTTTCCGGGCCGTGGAAAC-10 ACGTACAGTGATTTTAAATG GCATTGGTATCATTTCGACGGAGCGGACTGGGATGAATCCC-GGAAGATCAGCCGCATCTTTAAGTTTCGTGGGGAAGGAA AAGCGTGGGATTGGGAAGTAT-CAAGTGAAAACGGCAACTATGACTATTTAATGTATGCTGATGTTGACTACGACCACCCT GATGTCGTGGCAGAGACAAAAAATGGGGTATCTGGTATGCGAATGAACTGTCATTAGACGG-CTTCCGTATTGATGCCGC CAAACATATTAAATTTTCATTTCTGCGTGATTGGGTTCAGG-18 CGGTCAGACAGGCGACGGGAAAAGAAATGTTTACGGTTG CGGAGTATTGGCAG-GATGTT CCGCTTCATTTCAATTTACAGGCGCTTCCTCACAAGGAGGCGGATATGATAT-GAGGCGTTTGCTGGACGGTACCGTTGT GTCCAGGCATCCGGAAAAGGCGGTTACATTTGT-TGAAAATCATGACACACCCGGGACAGTCATTGGAATCGACAGTCC AAACTTGGTTTAA-20 ACCCCTTGCATACCCCTTTATTTTGACAAGAGAATCCGGTTATCCTCAGGTGTTCTATGGG-GATATG TACGGGACAAAAGGGACATCGCCAAAGGAAATTCCCTCACTGAAAGATAATATA-GAGCCGATTTTAAAAGCGCGTAAGGA GTACGCATACGGGCCCCAGCACGATTATATTGAC-CACCCGGATGTGATCGGATGGACGAGGGGAAGGTGACAGCTCCGCCG ATCAGGTTTGGCCGCTTTAATCACGGACGGACCGGCGGATCAAAGCGGATGTATGCCGG-25 CCTGAAAAATGCCGGC GAGACATGGTATGACATAACGGGCAACCGTTCAGATACTGTAA-AAATCGGATCTGACGGCTGGGGAGAGTTTCATGTAAA CGATGGGTCCGTCTCCATTTAT-**GTTCAGAAATAA**

SEO ID No. 4

VNGTLMQYFEWYTPNDGQHWKRLQNDAEHLSDIGITAVWI
PPAYKGLSQSDNGYGPYDLYDLGEFQQKGTVRTKYGTKSE

LQDAIGSLHSRNVQVYGDVVLNHKAGADATEDVTAVEVNP
ANRNQETSEEYQIKAWTDFRFFGRGNTYSDFKWHWYHFDG
ADWDESRKISRIFKFRGEGKAWDWEVSSENGNYDYLMYAD
VDYDHPDVVAETKKWGIWYANELSLDGFRIDAAKHIKFSF
LRDWVQAVRQATGKEMFTVAEYWQNNAGKLENYLNKTSFN
QSVFDVPLHFNLQAASSQGGGYDMRRLLDGTVVSRHPEKA
VTFVENHDTQPGQSLESTVQTWFKPLAYAFILTRESGYPQ
VFYGDMYGTKGTSPKEIPSLKDNIEPILKARKEYAYGPQH
DYIDHPDVIGWTREGDSSAAKSGLAALITDGPGGSKRMYA
GLKNAGETWYDITGNRSDTVKIGSDGWGEFHVNDGSVSIY

25

SEQ ID No. 5

aaattcgatattgaaaacgattacaaataaaaattataatagacgtaaacgttcgagggtttgctccctttttactcttt ttatgcaatcgtttcccttaatttttttggaagccaaaccgtcgaatgtaacatttgattaagggggaagggcatt

aacgtttcaccgcatcattcgaaaaggatggatgttcctgctcgcgtttttgctcactgtctcgctgttctgcccaacag gacagcccgccaaggctGCCGCACCGT-25 TTAACGGCACCATGATGCAGTATTTTGAATGGTACTTGCCGGATGATGGCACG TTATGG-ACCAAAGTGGCCAATGAAGCCAACAACTTATCCAGCCTTGGCATCACCGCTCTTTGGCTG-CCGCCCGCTTACAA AGGAACAAGCCGCAGCGACGTAGGGTACGGAGTATACGACTTGTA-TGACCTCGGCGAATTCAATCAAAAAGGGACCGTCC GCACAAAATACGGAACAAAAGCTC-AATATCTTCAAGCCATTCAAGCCGCCGCCGCTGGAATGCAAGTGTACGCCGAT GTC-30 GTGTTCGACCATAAAGGCGGCGCTGACGGCACGGAATGGGTGGACGCCGTCGAAGTCAAT-CCGTCCGACCGCAACCA AGAAATCTCGGGCACCTATCAAATCCAAGCATGGACGAAATT-TGATTTTCCCGGGGGGGGACACCTACTCCAGCTTTA AGTGGCGCTGGTACCATTTTG-ACGGCGTTGATTGGGACGAAAGCCGAAAATTGAGCCGCATTTACAAATTCCGCGGCATC GGCAAAGCGTGGGATTGGGAAGTAGACACGGAAAACGAAACTATGACTACTTAATGTAT-35 GCCGACCTTGATATGGATCA TCCCGAAGTCGTGACCGAGCTGAAAAACTGGGGGAAATG-GTATOTCAACACAACGAACATTGATGGGTTCCGGCTTGATG CCGTCAAGCATATTAAGT-TCASTTTTTTCCTCATTGGTTGTCGTATGTGCGTTCTCAGACTGGCAAGCCGCTATTTACC

GGAACGATGTCTTTGTTTGA TGCCCCGTTACACAACAATTTTATACCGCTTCCAAATCAG
GGGGCGCATTTGATATGCGCACGTTAATGACCAATACTC TCATGAAAGATCAACCGACATTGGCCGTCACCTTCGTTGATAATCATGACACCGGAACCCGGCCAAGCGCTGCAGTCATGG GTCGACCCATGGTTCAAACCGTTGGCTTACGCCTTTATTCTAACTCGGCAGGAAGGATACCCGTGCGTCTTTTATGGTGA CTATTATGGCATTCCACAATATAACATTCCTTCGCTGAAAAGCAAAATCGATCCGCTCCTCATCGCGCGCAGGGATTATG CTTACGGAACGCAACATGATTATCTTGATCACTCCGACATCATCGGGTGGACAAGGGAAGGGGGCACTGAAAAACCAGGA TCCGGACTGGCCGCACTGATCACCGATGGGCCGGGAGGAAGCAAATGGATGTACGTTGGCAAACAACACGGTGGAAAAAGT GTTCTATGACCTTACCGGCAACCG10 GAGTGACACCGTCACCATCAACAGTGATGGATGGGGGGGAATTCAAAGTCAATGGCG GTTCGGTTTCGGTTTGGGTTCCTAGAAAAACGACCGTTTCTACCATCGCTCGGCCGATCACAACCCGACCGTGGACTGGT GAATTCGTCCGTTGGACCGAACCACGGTTGGTGGCATGGCCTTGA

tgcctgcga

15

SEQ ID No. 6

AAPFNGTMMQYFEWYLPDDGTLWTKVANEANNLSSLGITA

LWLPPAYKGTSRSDVGYGVYDLYDLGEFNQKGTVRTKYGT
KAQYLQAIQAAHAAGMQVYADVVFDHKGGADGTEWVDAVE
VNPSDRNQEISGTYQIQAWTKFDPPGRGNTYSSPXWRWYH
FDGVDWDESRKLSRIYKFRGIGKAWDWEVDTENGNYDYLM
YADLDMDHPEVVTELKNWGKWYVNTTNIDGFRLDAVKHIK
FSFFFDWLSYVRSQTGKPLPTVGEYWSYDINKLHNYITKT
DGTMSLFDAPLHNKFYTASKSGGAFDMRTLMTNTLMKDQP
TLAVTFVDNHDTEPGQALQSWVDPWFKPLAYAFILTRQEG
YPCVFYGDYYGIPQYNIPSLKSKIDPLLIARRDYAYGTQH
DYLDHSDIIGWTREGGTEKPGSGLAALITDGPGGSKWMYV
GKQHAGKVFYDLTGNRSDTVTINSDGWGEFKVNGGSVSVW
VPRKTTVSTIARPITTRPWTGEFVRWTEPRLVAW

SEQ ID No. 10

- 38 1 ATPADWRSQS IYFLLTDRFA RTDGSTTATC
 - 31 NTADOKYCGG TWQGIIDKLD YIQGMGFTAI
 - 61 WITPVTAOLP OTTAYGDAYH GYWQQDIYSL
 - 91 NENYGTADDL KALSSALHER GMYLMVDVVA

	121	NHMGYDGAGS	SVDYSVFKPF	SSQDYFHPFC
	151	FIQNYEDQTQ	VEDCWLGDNT	VSLPDLDTTK
	181	DAAKMEMADM	VGSLVSNYSI	DGLRIDTVKH
	211	VQKDFWPGYN	KAAGVYCIGE	VLDGDPAYTC
5	241	PYQNVMDGVL	NYPIYYPLLN	AFKSTSGSMD
	271	DLYNMINTVK	SDCPDSTLLG	TFVENHDNPR
	301	FASYTNDIAL	AKNVAAFIIL	NDGIPIIYAG
	331	QEQHYAGGND	PANREATWLS	GYPTDSELYK
	361	LIASANAIRN	YAISKDTGFV	TYKNWPIYKD
20	391	DITIAMRKGT	DGSQIVTILS	nkgasgdsyt
	421	LSLSGAGYTA	GQQLTEVIGC	TTVTVGSDGN
	451	VPVPMAGGLP	RVLYPTEKLA	GSKICSSS

ころれらまたのまたないたみんそのみなんなほどとなかないはないがんかんかんかんかんなんなだ ちんとう <u>计设定性效应计划设定处理效应处理效应性的现代的现代的数据数据数据数据数据数据数据数据数据数据数据数据数据数据数据数据数据</u> బర్జ్ఎం…న.*నట్రెడ్డ్ ఎం.ఇన.ఎం.ఇనట్రెడ్డ్ ఆం.ఇనట్రెడ్డ్ ఎం.ఇనట్రెడ్డ్ ఎం.ఇనట్రెడ్డ్ ఎం.ఇన **まするなければははなけれるないないないないないないないままままままなみなくなくなくないない。まするなければはないまままないかまままながれまるない。まないなりはまままない。**

| March | Marc

| Color | Colo

| Color | Colo

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| Color | Colo

| Mar. |

| March | Marc

| Miles | Mile

| 17.00 | 7.75 | 0. W. M. A 1000 | 35. 002 | 37. 017 | 37. 505 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7.75 | 100 | 6.06 | 7

| Color | Colo

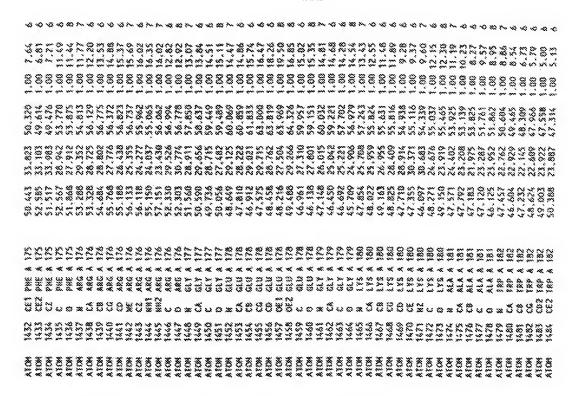
| Color | Colo

| March | Marc

| Marie | 1220 | C. | 189 | M. | 153 | C. | 1541 | C. | 1875 | S. | 1875 | D. | 198 | S. | 1875 | C. | 1875 | M. |

| 16.7 | 16.7 | 16.8 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 | 12.4 |

| 1276 | 1270 | 1270 | 115 | 1157 | 1276 | 136. 136 | 137. 100 | 1370 | 100 | 15.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 | 14.00 |



Appendix 1

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东京文文文文表表思思于了文文表表的的文文文文表现现象介述的文文的文文结片组织结合更具是表示文品表表现的设计分词的结似的编码的名词形式对对外分别的对例对的对外或的研究的目的用明显写明计处析的对象的的结实识别的特殊的对例的
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చేవిది ఆంజని బెక్టిన్ ప్రధాని నిని ప్రత్యేషం అండి పలు ఉనే బిక్టిన్ పలు ఉనే బిక్టిన్ ప్రస్తేషన్ ఇంజనే బిక్టిన్ త మారాలు

PCT/DK96/00057

| 1645 | 184 | 0 | 1859 | 200 | 45.308 | 31.455 | 47.481 | 1.00 | 5.00 | 7.00 | 7.00 | 1.00 | 1.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 | 7.00 |

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| 1856 | C. | ASP | A 226 | 19.35 | 35.82 | 35.82 | 1.00 | 7.75 | 35.82 | 4.00 | 7.75 | 35.82 | 4.00 | 7.75 | 35.82 | 4.00 | 7.75 | 35.82 | 4.00 | 7.75 | 35.82 | 4.00 | 7.75 | 35.82 | 4.00 | 7.75 | 35.82 | 4.00 | 7.75 | 35.82 | 4.00 | 7.75 | 35.82 | 4.00 | 7.75 | 35.82 | 4.00 | 7.75 | 35.82 | 4.00 | 7.75 | 35.82 | 4.00 | 7.75 | 35.82 | 4.00 | 7.75 | 35.82 | 4.00 | 7.75 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 | 35.82 |

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| Miles | 2519 | Co. S.E.R | 277 | 4.0 72 | 13. 13. 13. 13. 13. 15 | 100 10.25. 15 | 10. 150 | 1.00 10.25. 15 | 10. 150 | 1.00 10.25. 15 | 10. 150 | 1.00 10.25. 15 | 10. 150 | 1.00 10.25. 15 | 10. 150 | 1.00 10.25. 15 | 10. 150 | 1.00 10.25. 15 | 10. 150 | 1.00 10.25. 15 | 10. 150 | 1.00 10.25. 15 | 10. 150 | 1.00 10.25. 15 | 10. 150 | 1.00 10.25. 15 | 10. 150 | 1.00 10.25. 15 | 10. 150 | 1.00 10.25. 15 | 10. 150 | 1.00 10.25. 15 | 10. 150 | 1.00 10.25. 15 | 10. 150 | 1.00 10.25. 15 | 10. 150 | 1.00 10.25. 15 | 10. 150 | 1.00 10.25. 15 | 10. 150 | 1.00 10.25. 15 | 10. 150 | 1.00 10.25. 15 | 10. 150 | 1.00 10.25. 15 | 10. 150 | 1.00 10.25. 15 | 10. 150 | 1.00 10.25. 15 | 10. 150 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15 | 1.00 10.25. 15

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Miles	2551	Oct	ASP	A 125	38, 076	25, 409	1400	2746	8	
Miles	2555	COL	ASP	A 275	37, 102	27, 409	1,00	1,00	9, 34	8
Miles	2555	COL	ASP	A 275	37, 102	27, 438	1,10	1,00	9, 34	8
Miles	2555	COL	ASP	A 235	35, 117	27, 238	11,254	1,00	9, 34	8
Miles	2555	COL	ASP	A 235	35, 100	27, 435	21, 235	1,00	1,00	1,35
Miles	2555	COL	ASP	A 235	35, 100	27, 435	22, 435	3, 100	9, 24	
Miles	2555	COL	ASP	A 235	35, 100	27, 435	22, 435	3, 100	1, 25	
Miles	2555	COL	ASP	A 235	35, 100	27, 435	27, 435	30, 100	1, 25	
Miles	2555	COL	ASP	A 235	35, 100	27, 435	27, 435	30, 100	1, 25	
Miles	2555	COL	ASP	A 235	35, 100	27, 435	30, 100	1, 25		
Miles	2555	COL	ASP	A 235	35, 100	27, 435	30, 100	1, 25		
Miles	2555	COL	ASP	A 235	37, 125	27, 435	30, 100	1, 25		
Miles	2555	COL	ASP	A 235	37, 125	27, 435	37, 20	30, 100	1, 25	
Miles	2555	COL	ASP	A 235	37, 125	27, 435	37, 20	30, 100	1, 25	
Miles	2555	COL	ASP	A 235	37, 20	37, 20	37, 30	37, 43		
Miles	2555	COL	ASP	A 235	37, 20	37, 30	37, 30	37, 30		
Miles	2555	COL	ASP	A 235	37, 30	37, 30	37, 30	37, 30		
Miles	2557	COL	ASP	A 235	37, 30	37, 30	37, 30	37, 30		
Miles	2557	COL	ASP	A 235	37, 30	37, 30	37, 30	37, 30		
Miles	2557	COL	ASP	A 235	37, 30	37, 30	37, 30	37, 30		
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Miles	2557	COL	ASP	A 235	37, 30	37, 30	37, 30	37, 30		
Miles	2557	COL	AS							

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| Marie | 2810 | 8 | 780 | 8 | 355 | 31 637 | 23 1866 | 10.213 | 1.00 | 9.313 | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  | 5.8  |
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1777 | E22 Will # 139 | 38.74 | 21.841 | 16.483 | 1.00 | 9.06 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14 | 4.14

| ALICA | 266.5 | CO | 110 | A 351 | 27.589 | 22.986 | 19.575 | 100 | 2.426 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 | 5.441 |

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| Marie | 1500 | C. D. PRO A C.T. | 15, 409 | 10, 665 | 0, 1084 | 1, 600 | 14, 646 | 5, 4173 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1, 1015 | 1

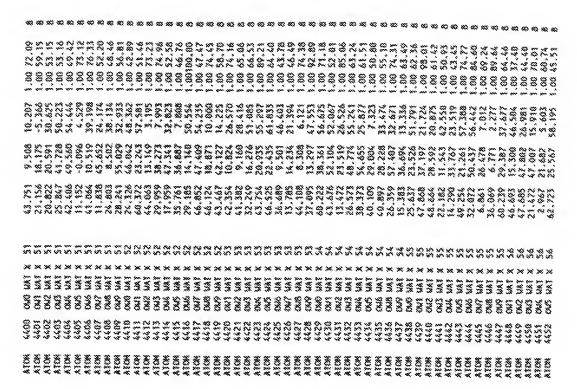
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CLAIMS

 A method of constructing a variant of a parent Termamyl-like α-amylase, which variant has α-amylase activity and at least one altered property as compared to said parent α-amylase, which method comprises

i) analysing the structure of the parent Termamyl-like αamylase to identify at least one amino acid residue or at least
to one structural part of the Termamyl-like α-amylase structure,
which amino acid residue or structural part is believed to be
of relevance for altering said property of the parent Termamyllike α-amylase (as evaluated on the basis of structural or
functional considerations),

18

ii) constructing a Termamyl-like α -amylase variant, which as compared to the parent Termamyl-like α -amylase, has been modified in the amino acid residue or structural part identified in i) so as to alter said property, and

- iii) testing the resulting Termamyl-like α -amylase variant for said property.
- 2. The method according to claim 1, wherein the property to be altered is selected from the group consisting of substrate specificity, substrate binding, substrate cleavage pattern. temperature stability, pH dependent activity, pH dependent stability (especially increased stability at low (e.g. pH<6) or high (e.g. pH>9) pH values), stability towards oxidation, Ca²⁻dependency and specific activity.
- 3. The method according to claim 1 or 2, wherein the property to be altered is the calcium ion dependency and the structural part to be modified is selected from the group consisting of the C domain, the interface between the A and B domain, the interface between the A and B domain, the interface between the interaction to a calcium binding site of the Termamyl-like o-amylase.

4. The method according to claim 1 or 2, wherein the property to be altered is the substrate cleavage pattern and the structural part to be modified is located within 10Å from an amino acid residue of the substrate binding site.

- 5. A method of constructing a variant of a parent Termamyl-like α -amylase, which variant has α -amylase activity and one or more altered properties as compared to said parent α -amylase, which method comprises
- 10 i) comparing the three-dimensional structure of the Termanyl-like α -amylase with the structure of a non-Termanyl-like α -amylase,
 - ii) identifying a part of the Termamyl-like α -amylase structure which is different from the non-Termamyl-like α -amylase
- is structure and which from structural or functional considerations is contemplated to be responsible for differences in one or more properties of the Termamyl-like and non-Termamyl-like a-amylase, and
- iii) modifying the part of the Termamyl-like α -amylase identified in ii) whereby a Termamyl-like α -amylase variant is obtained, one or more properties of which differ from the parent Termamyl-like α -amylase.
- 6. The method according to claim 6, wherein, in step iii), the 25 part of the Termamyl-like α -amylase is modified so as to ressemble the corresponding part of the non-Termamyl-like α -amylase.
- 7. The method according to claim 5 or 6, wherein, in step iii),
 the modification is accomplished by deleting one or more amino acid residues of the part of the Termamyl-like α-amylase to be modified; by replacing one or more amino acid residues of the part of the Termamyl-like α-amylase to be modified with the amino acid residues occupying corresponding positions in the non-Termamyl-like α-amylase; or by insertion of one or more amino acid residues present in the non-Termamyl-like α-amylase into a corresponding position in the Termamyl-like α-amylase.

- 8. The method according to any of claims 5-7, wherein the non-Termamyl-like α -amylase structure is the structure of a fungal α -amylase or a mammalian α -amylase.
- 5 9. The method according to claim 8, wherein the non-Termamyllike α-amylase is the Aspergillus oryzae TAKA α-amylase, the A. niger acid α-amylase, the Bacillus subtilis α-amylase or the pig pancreatic α-amylase.
- 10 10. The method according to any of claims 1-9, wherein the parent Termamyl-like α-amylase is derived from a strain of Bacillus.
- 11. The method according to claim 10, wherein the parent o15 amylase is derived from a strain of a B. licheniformis, B. amyloliquefaciens, B. stearothermophilus or a strain from an
 alkalophilic Bacillus sp. such as NCIB 12289, NCIB 12512 or
 NCIB 12513.
- 20 12. The method according to any of claims 1-11, wherein the parent α -amylase is a hybrid α -amylase comprising a combination of partial amino acid sequences derived from at least two α -amylases, of which one is a Termamyl-like α -amylase and the other(s) are, e.g., from a microbial and/or a mammalian α -25 amylase.
- 13. The method according to any of claims 5-12, wherein the part of the parent Termamyl-like α-amylase to be modified and identified in step ii) is loop 1, loop 2, loop 3 and/or loop 8 so of the parent α-amylase.
 - 13. A method of constructing a variant of a parent Termamyllike α -amylase, which has a decreased calcium ion dependency as compared to said parent, which method comprises:
 - i) identifying an amino acid residue within 10Å from a Ca binding site of a Termamyl-like α -amylase in a model of the three-dimensional structure of said α -amylase, which from

structural or functional considerations is believed to be responsible for a non-optimal calcium ion interaction.

- ii) constructing a variant in which said amino acid residue is replaced with another amino acid residue which from structural
- s or functional considerations is believed to be important for establishing a higher Ca" binding affinity, and
 - iii) testing the $Ca^{2^{\ast}}$ dependency of the resulting Termamyl-like α -amylase variant.
- 10 14. A method of constructing a variant of a parent Termamyllike α -amylase which variant has α -amylase activity and an altered pH dependent activity, which method comprises
- i) in a three-dimensional structure of the Termamyl-like α 15 amylase in question, identifying an amino acid residue within
 15Å from an active site residue, in particular 10Å from an
 active site residue, which amino acid residue is contemplated
 to be involved in electrostatic or hydrophobic interactions
 with an active site residue.

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- ii) replacing, in the structure, said amino acid residue with an amino acid residue which changes the electrostatic and/or hydrophobic surroundings of an active site residue and evaluating the accompodation of the amino acid residue in the structure.
 - iii) optionally repeating step i) and/or ii) until an amino acid replacement has been identified which is accompdated into the structure.

- iv) Constructing a Termamyl-like α -amylase variant resulting from steps i), ii) and optionally iii) and testing the pH dependent activity of said variant.
- 35 15. A method of increasing the thermostability and/or altering the temperature optimum of a parent Termamyl-like α-amylase, which method comprises

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- i) identifying an internal hole or a crevice of the parent Termamyl-like α -amylase in the three-dimensional structure of said α -amylase,
- ii) replacing, in the structure, one or more amino acid s residues in the neighbourhood of the hole or crevice identified in i) with another amino acid residue which from structural or functional considerations is believed to increase the hydrophobic interaction and to fill out or reduce the size of the hole or crevice,
- 10 iii) constructing a Termamyl-like o-amylase variant resulting from step ii) and testing the thermostability and/or temperature optimum of the variant.
- 16. A method of constructing a variant of a Termamyl-like α-15 amylase which has a reduced ability to cleave a substrate close to the branching point, which method comprises
- identifying the substrate binding area of the parent Termamyl-like α-amylase in a model of the three-dimensional structure of said α-amylase,
- ii) replacing, in the model, one or more amino acid residues of the substrate binding area of the cleft identified in i), which is/are believed to be responsible for the cleavage pattern of the parent o-amylase, with another amino acid residue which from structural considerations is believed to result in an altered substrate cleavage pattern, or deleting one or more amino acid residues of the substrate binding area contemplated to introduce favourable interactions to the substrate binding area contemplated to introduce favourable interactions to the substrate binding area contemplated to introduce favourable interactions to the substrate binding area substrate.
- iii) constructing a Termamyl-like a-amylase variant resulting from step ii) and testing the substrate cleavage pattern of the variant.
 - 17. The method according to any of the preceeding claims, in which the q-amylase variant is obtained by cultivating a

o-amylase as a template.

microorganism comprising a DNA sequence encoding the variant under conditions which are conducive for producing the variant, and optionally subsequently recovering the variant from the resulting culture broth.

- 18. A variant of a parent Termamyl-like a-amylase, in which variant at least one amino acid residue of the parent a-amylase, which is/are present in a fragment corresponding to the amino acid fragment 44-57 of the amino acid sequence of SEQ ID No. 4, has been deleted or replaced with one or more amino acid residues which is/are present in a fragment corresponding to the amino acid fragment 66-84 of the amino acid sequence shown in SEQ ID No. 10, or in which one or more additional amino acid residues has been added using the relevant part of SEQ ID No. 10 or a corresponding part of another Fungamyl-like
- 19. A variant of a parent Termamyl-like α-amylase, which variant has a region which, when the amino acid sequence of variant is aligned most closely with the amino acid sequence of the said parent α-amylase, occupies the same position as the portion from residue X to residue Y of SEQ ID No 4, the said region having at least 80% sequence homology with the part of SEQ ID No 10 extending from residue Z to residue V of SEQ ID No 25 10, wherein
 - X is the amino acid residue occupying position 44, 45, 46, 47 or 48 of SEQ ID No. 4,
 - Y is the amino acid residue occupying position 51, 52, 53, 54, 55, 56 or 57 of SEQ ID No. 4,
- 30 Z is the amino acid residue occupying position 66, 67, 68, 69 or 70 of SEQ ID No. 10, and
 V is the amino acid residue occupying position 78, 79, 80, 81, 82, 83 or 84 of SEQ ID No. 10.
- 15 20. The variant according to claim 18 or 19, wherein X is the amino acid residue occupying position 48 and Y the amino acid residue occupying position 51 of SEQ ID NO 4 and Z is the amino

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acid residue occupying position 70 and V the amino acid residue occupying position 78 in SEQ ID No 10.

- 21. A variant of a parent Termamyl-like α-amylase, in which variant at least one of the amino acid residues of the parent α-amylase, which is/are present in an amino acid fragment corresponding to the amino acid fragment 195-202 of the amino acid sequence of SEQ ID No. 4, has been deleted or replaced with one or more of the amino acid residues which is/are present in an amino acid fragment corresponding to the amino acid fragment 165-177 of the amino acid sequence shown in SEQ ID No. 10, or in which one or more additional amino acid residues has been added using the relevant part of SEQ ID No. 10 or a corresponding part of another Fungamyl-like α-amylase as a template.
- 22. A variant of a parent Termamyl-like α-amylase, which variant has a region which, when the amino acid sequence of variant is aligned most closely with the amino acid sequence of the said parent α-amylase, occupies the same position as the portion from residue X to residue Y of SEQ ID No 4, the said region having at least 80%, such as 90% sequence homology with the part of SEQ ID No 10 extending from residue Z to residue V of SEQ ID No 10, wherein
- 25 X is the amino acid occupying position 195 or 196 of SEQ ID No. 4,
 - Y is the amino acid residue occupying position 198, 199, 200, 201, or 202 of SEQ ID No. 4,
 - Z is the amino acid residue occupying position 165 or 166 of SEQ ID No. 10, and
- V is the amino acid residue occupying position 173, 174, 175, 176 or 177 of SEQ ID No. 10.
 - 23. The variant according to claim 21 or 22, in which the amino acid fragment α^{μ} the parent α -amylase, which corresponds to

amino acid residues 196-198 of SEQ ID No. 4, has been replaced with the amino acid fragment corresponding to amino acid residues 166-173 of the amino acid sequence shown in SEQ ID No. 10.

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- 24. A variant of a parent Termamyl-like α-amylase, in which variant at least one of the amino acid residues of the parent α-amylase, which is/are present in a fragment corresponding to the amino acid fragment 117-185 of the amino acid sequence of SEQ ID No. 4, has/have been deleted or replaced with one or more of the amino acid residues, which is/are present in an amino acid fragment corresponding to the amino acid fragment 98-210 of the amino acid sequence shown in SEQ ID No. 10, or in which one or more additional amino acid residues has been added using the relevant part of SEQ ID No. 10 or a corresponding part of another Fungamyl-like α-amylase as a template.
- 25. A variant of a parent Termamyl-like α-amylase, which variant has a region which, when the amino acid sequence of variant is aligned most closely with the amino acid sequence of the said parent α-amylase, occupies the same position as the portion from residue X to residue Y of SEQ ID No 4, the said region having at least 80%, such as at least 90% sequence homology with the part of SEQ ID No 10 extending from residue Z5 Z to residue V of SEQ ID No 10, wherein

% is the amino acid occupying position 117, 118, 119, 120 or 121 of SEQ ID No. 4,

- 30 Y is the amino acid occupying position 181, 182, 183, 184 or 185 of SEQ ID No. 4,
 - Z is the amino acid occupying position 98, 99, 100, 101, 102 of SEQ ID No. 10, and
 - V is the amino acid occupying position 206, 207, 208, 209 or 210 of SEQ ID No. 10.

26. The variant according to claim 24 or 25, in which an amino acid fragment of the parent σ-amylase, which corresponds to amino acid residues 121-181 of SEQ ID No. 4, has been replaced with the amino acid fragment corresponding to amino acid residues 102-206 of the amino acid sequence shown in SEQ ID No. 10.

27. A variant of a parent Termamyl-like α-amylase, in which variant at least one of the amino acid residues of the parent α-amylase, which is/are present in a fragment corresponding to the amino acid fragment 117-181 of the amino acid sequence of SEQ ID No. 4, has/have been deleted or replaced with one or more of the amino acid residues, which is/are present in an amino acid fragment corresponding to the amino acid fragment to 98-206 of the amino acid sequence shown in SEQ ID No. 10, or in which one or more additional amino acid residues has been added using the relevant part of SEQ ID No. 10 or a corresponding part of another Fungamyl-like α-amylase as a template.

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28. A variant of a parent Termamyl-like α-amylase, which variant has a region which, when the amino acid sequence of variant is aligned most closely with the amino acid sequence of the said parent α-amylase, occupies the same position as the portion from residue X to residue Y of SEQ ID No 4, the said region having at least 80%, such as at least 90% sequence homology with the part of SEQ ID No 10 extending from residue Z to residue V of SEQ ID No 10, wherein

X is the amino acid occupying position 117, 118, 119, 120 or 30 121 of SEQ ID No. 4,

Y is the amino acid occupying position 174, 175, 176 or 177 of SEQ ID No. 4,

Z is the amino acid occupying position 98, 99, 100, 101, 102 of SEO ID No. 10, and

V is the amino acid occupying position 199, 200, 201 or 202 of SEQ ID No. 10.

29. The variant according to claim 27 or 28, in which the amino sacid fragment of the parent α-amylase, which corresponds to amino acid residues 121-174 of SEQ ID No. 4, has been replaced with the amino acid fragment corresponding to amino acid residues 102-199 of the amino acid sequence shown in SEQ ID No. 10.

- 30. A variant of a parent Termamyl-like α-amylase, in which variant at least one of the amino acid residues of the parent α-amylase, which is/are present in an amino acid fragment corresponding to the amino acid fragment 12-19 of the amino 15 acid sequence of SEQ ID No. 4, has/have been deleted or replaced with one or more of the amino acid residues, which is/are present in an amino acid fragment which corresponds to the amino acid fragment 28-42 of SEQ ID No. 10, or in which one or more additional amino acid residues has/have been inserted using the relevant part of SEQ ID No. 10 or a corresponding part of another Fungamyl-like α-amylase as a template.
- 31. A variant of a parent Termamyl-like \$\alpha\$-amylase, which variant has a region which, when the amino acid sequence of variant is aligned most closely with the amino acid sequence of the said parent \$\alpha\$-amylase, occupies the same position as the portion from residue X to residue Y of SEQ ID No 4, the said region having at least 80%, such as at least 90% sequence homology with the part of SEQ ID No 10 extending from residue Z to residue V of SEQ ID No 10, wherein
- X is the amino acid occupying position 12, 13 or 14 of SEQ ID No. 4.
 - Y is the amino acid occupying position 15, 16, 17, 18 or 19 of SEQ ID No. 4,
- 35 Z is the amino acid occupying position 28, 29, 30, 31 or 32 of SEQ ID No. 10, and
 - V is an amino acid residue corresponding to the amino acid occupying position 38, 39, 40, 41 or 42 of SEQ ID No. 10.

- 32. The variant according to claim 30 or 31, in which the amino acid fragment of the parent α-amylase, which corresponds to amino acid residues 14-15 of SEQ ID No. 4, has been replaced with the amino acid fragment corresponding to amino acid residues 32-38 of the amino acid sequence shown in SEQ ID No. 10.
- 33. A variant of a parent Termamyl-like α-amylase, in which variant at least one of the amino acid residues of the parent α-amylase, which is present in a fragment corresponding to amino acid residues 7-23 of the amino acid sequence of SEQ ID No. 4, has/have been deleted or replaced with one or more amino acid residues, which is/are present in an amino acid fragment corresponding to amino acid residues 13-45 of the amino acid sequence shown in SEQ ID No. 10, or or in which one or more additional amino acid residues has/have been inserted using the relevant part of SEQ ID No. 10 or a corresponding part of another Fungamyl-like α-amylase as a template.
- 20 34. A variant of a parent Termamyl-like α-amylase, which variant has a region which, when the amino acid sequence of variant is aligned most closely with the amino acid sequence of the said parent α-amylase, occupies the same position as the portion from residue X to residue Y of SEQ ID No 4, the said 25 region having at least 80%, such as at least 90% sequence homology with the part of SEQ ID No 10 extending from residue Z to residue V of SEQ ID No 10, wherein X is the amino acid occupying position 7 or 8 of SEQ ID No. 4,
- y is the amino acid occupying position 18, 19, 20, 21, 22 or 23 of SEQ ID No. 4,
 - Z is the amino acid occupying position 13 or 14 of SEQ ID No. 10, and
- V is the amino acid occupying position 40, 41, 42, 43, 44 or 45 of SEQ ID No. 10.

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- 35. The variant according to claim 33 or 34, in which the amino acid fragment of the parent o-amylase, which corresponds to amino acid residues 8-18 of SEQ ID No. 4, has been replaced with the amino acid fragment corresponding to amino acid residues 14-40 of the amino acid sequence shown in SEQ ID No. 10.
- 36. A variant of a parent Termamyl-like α-amylase, in which variant at least one of the amino acid residues of the parent α-amylase, which is present in a fragment corresponding to amino acid residues 322-346 of the amino acid sequence of SEQ ID No. 2, has/have been deleted or replaced with one or more amino acid residues, which is/are present in an amino acid fragment corresponding to amino acid residues 291-313 of the amino acid sequence shown in SEQ ID No. 10, or or in which one or more additional amino acid residues has/have been inserted using the relevant part of SEQ ID No. 10 or a corresponding part of another Fungamyl-like α-amylase as a template.
- 20 37. A variant of a parent Termamyl-like α-amylase, which variant has a region which, when the amino acid sequence of variant is aligned most closely with the amino acid sequence of the said parent α-amylase, occupies the same position as the portion from residue X to residue Y of SEQ ID No 2, the said region having at least 80% sequence homology with the part of SEQ ID No 10 extending from residue Z to residue V of SEQ ID No 10, wherein

X is the amino acid occupying position 322, 323, 324 or 325 of SEQ ID No. 2,

Y is the amino acid occupying position 343, 344, 345 or 346 of SEQ ID No. 2,

Z is the amino acid occupying position 291, 292, 293 or 294 of 35 SEQ ID No. 10, and

V is the amino acid occupying position 310, 311, 312 or 313 of SEO ID No. 10.

38. The variant according to claim 36 or 37, in which the amino acid fragment of the parent α-amylase, which corresponds to amino acid residues 325-345 of SEQ D No. 2, has been replaced with the amino acid fragment corresponding to amino acid residues 294-313 of the amino acid sequence shown in SEQ ID No. 10.

39. A variant of a parent Fungamyl-like α-amylase, in which variant at least one of the amino acid residues of the parent α-amylase, which is/are present in an amino acid fragment corresponding to amino acid residues 291-313 of the amino acid sequence of SEQ ID No. 10, has/have been deleted or replaced with one or more of the amino acid residues, which is/are present in an amino acid fragment corresponding to amino acid residues 98-210 of the amino acid sequence shown in SEQ ID No. 4, or in which one or more additional amino acid residues has/have been inserted using the relevant part of SEQ ID No. 4 or a corresponding part of another Termamyl-like α-amylase as a template.

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40. A variant of a parent Fungamyl-like α-amylase, which variant has a region which, when the amino acid sequence of variant is aligned most closely with the amino acid sequence of the said parent α-amylase, occupies the same position as the portion from residue X to residue Y of SEQ ID No 10, the said region having at least 80%, such as at least 90% sequence homology with the part of SEQ ID No 10 extending from residue Z to residue V of SEQ ID No 4, wherein

X is the amino acid occupying position 117, 118, 119, 120 or 30 121 of SEQ ID No. 10,

Y is the amino acid occupying position 181, 182, 183, 184 or 185 of SEQ ID No. 10,

38 Z is the amino acid occupying position 98, 99, 100, 101 or 102 of SEQ ID No. 4, and

V is the amino acid occupying position 206, 207, 208, 209 or 210 of SEQ ID No. 4.

41. The variant according to claim 39 or 40, in which the amino 5 acid fragment of the parent α-amylase, which corresponds to amino acid residues 121-181 of SEQ ID No. 10, has been replaced with the amino acid fragment corresponding to amino acid residues 102-206 of the amino acid sequence shown in SEQ ID No. 4.

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- 42. A variant according to any of claims 39-41, in which the the amino acid fragment of the parent α-amylase, which corresponds to amino acid residues 121-174 of SEQ ID No. 10, has been replaced with the amino acid fragment corresponding to 15 amino acid residues 102-199 of the amino acid sequence shown in SEQ ID No. 4.
- 43. A variant of a parent Fungamyl-like α-amylase, in which an amino acid fragment corresponding to amino acid residues 181 20 184 of the amino acid sequence shown in SEQ ID No. 10 has been deleted.
- 45. A variant of a parent Termamyl-like α -amylase, which exhibits α -amylase activity and which has a decreased Ca^{2n} dependency as compared to the parent α -amylase.
 - 46. A variant according to claim 45, which comprises a mutation in a position corresponding to at least one of the following positions in SEQ ID NO 2:
- 30 N104, A349, I479, L346, I430, N457, K385, F350, I411, H408 or G303, in particular a mutation corrsponding to N104D;

A349C+I479C:

L346C+I430C:

35 N457D, E;

N457D, E+K385R;

F350D, E+I430R, K;

F350D, E+1411R, K:

H408Q,E,N,D; and/or G303N,D,Q,E.

47. A variant of a parent Termamyl-like α-amylase which sexhibits a higher activity below the pH optimum than the parent α-amylase, which variant comprises a mutation of an amino acid residue corresponding to at least one of the following positions of the B. licheniformis α-amylase (SEQ ID NO 2): E336, Q333, P331, I236, V102, A232, I103, L196, in particular at least one of the following mutations:

E336R, K; Q333R, K; P331R, K; V102R, K, A, T, S, G; 1236K, R, N; 15 1103K, R; L196K, R; and/or A232T, S, G.

48. A variant of a parent Termamyl-like α-amylase which 20 exhibits a higher activity above the pH optimum than the parent α-amylase, which variant comprises a mutation of an amino acid residue corresponding to at least one of the following positions of the β. licheniformis α-amylase (SEQ ID NO 2): N236, H281 and/or Y273, in particular one of the follwoing 25 mutations:

N326I,Y,F,L,V; H281F,I,L; and/or Y273F,W.

49. A variant of a parent Termamyl-like α-amylase which exhibits α-amylase activity and which has an increased thermostability and/or altered temperature optimum as compared to the parent α-amylase, which variant comprises a mutation of an amino acid residue corresponding to at least one of the following positions of the B. licheniformis α-amylase (SEQ ID NO 2):

```
L61, Y62, F67, K106, G145, I212, S151, R214, Y150, F143, R146,
   L241, I236, L7, V259, F284, F350, F343, L427 and/or V481, in
   particular at least one of the following mutations:
   L61W, V, F;
 5 Y62W;
   F67W:
   K106R, F, W;
   G145F, W
   I212F, L, W, Y, R, K;
10 S151 replaced with any other amino acid residue and in
   particular with F,W,I or L;
   R214W;
   YISOR, K:
   F143W:
15 R146W:
  L2411, F, Y, W;
   1236L, F, W, Y:
  L7F. I.W:
  V259F, I, L;
20 F284W;
  F350W;
  F343W;
  L427F, L, W: and/or
  V481.F.I.L.W.
25
   50. A variant of a parent Termamyl-like \alpha-amylase, which
  exhibits a-amylase activity and which has a reduced capability
  of cleaving an oligo-saccharide substrate close to the
  branching point as compared to the parent \alpha-amylase, which
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V54, D53, Y56, Q333 and/or G57, in particular at least one of the following mutations:
V54L,I,F,Y,W,R,K,H,E,Q;
D53L,I,F,Y,W;

yo variant comprises a mutation of an amino acid residue corresponding to at least one of the following positions of the

B. licheniformis α-amylase (SEQ ID NO 2):

Y,56W;

0333W; and/or G57 to all possible amino acid residues.

- 51. The variant according to any of claims 17-50, wherein one s or more proline residues present in the amino acid residues with which the parent o-amylase is modified are replaced with a non-proline residue such as alanine.
- 52. The variant according to any of claims 17-51, wherein one 10 or more cysteine residues present in the amino acid residues with which the parent o-amylase is modified are replaced with a non-cysteine residue such as alanine.
- 53. A DNA construct comprising a DNA sequence encoding an α-15 amylase variant according to any of claims 17-52.
 - 54. A recombinant expression vector which carries a DNA construct according to Claim 53.
- 20 55. A cell which is transformed with a DNA construct according to Claim 53 or a vector according to Claim 54.
 - 56. A cell according to Claim 55, which is a microorganism.
- 25 57. A cell according to Claim 56, which is a bacterium or a fungus.
 - 58. The cell according to Claim 57, which is a grampositive bacterium such as Bacillus subtilis, Bacillus licheniformis,
- 30 Bacillus lentus, Bacillus brevis, Bacillus stearothermophilus. Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus coagulans, Bacillus circulans, Bacillus lautus or Bacillus thuringiensis.
- 35 59. Use of an α -amylase variant according to any of claims 17-52 for washing and/or dishwashing.

- 60. Use of an α -amylase variant according to any of claims 17-52 for desizing.
- 61. Use of an α -amylase variant according to any of claims 17-5 2 for starch liquefaction.
 - 62. A detergent additive comprising an α -amylase variant according to any of claims 17-52, optionally in the form of a non-dusting granulate, stabilised liquid or protected enzyme.
- 63. A detergent additive according to Claim 62 which contains 0.02-200 mg of enzyme protein/g of the additive.
- 64. A detergent additive according to Claim 62 or 63, which 15 additionally comprises another enzyme such as a protease, a lipase, a peroxidase, another amylolytic enzyme and/or a cellulase.
- 65. A detergent composition comprising an α -amylase variant according to any of claims 17-52.
 - 66. A detergent composition according to Claim 65 which additionally comprises another enzyme such as a protease, a lipase, a peroxidase, another amylolytic enzyme and/or a cellulase.
 - 67. A manual or automatic dishwashing detergent composition comprising an α-amylase variant according to any of claims 17-52.
- 30 68. A dishwashing detergent composition according to Claim 67 which additionally comprises another enzyme such as a protease, a lipase, a peroxidase, another amylolytic enzyme and/or a cellulase.
- 35 69. A manual or automatic laundry washing composition comprising an α-amylase variant according to any of claims 17-52.

70. A laundry washing composition according to Claim 69, which additionally comprises another enzyme such as a protease, a lipase, a peroxidase, an amylolytic enzyme and/or a cellulase.

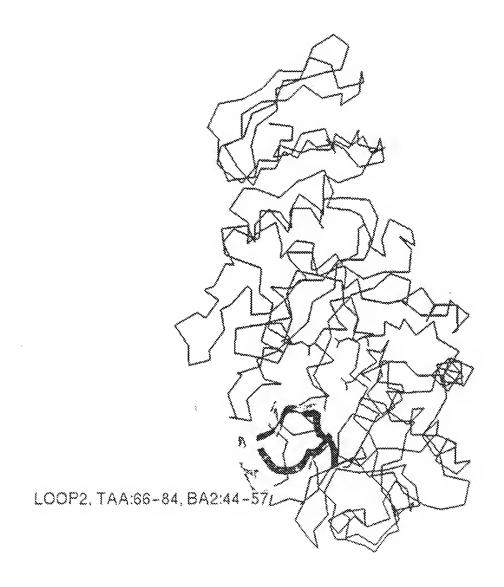


Fig. 1

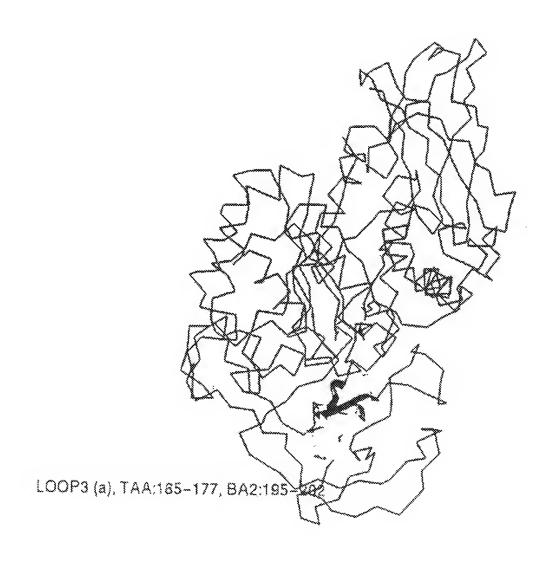


Fig. 2

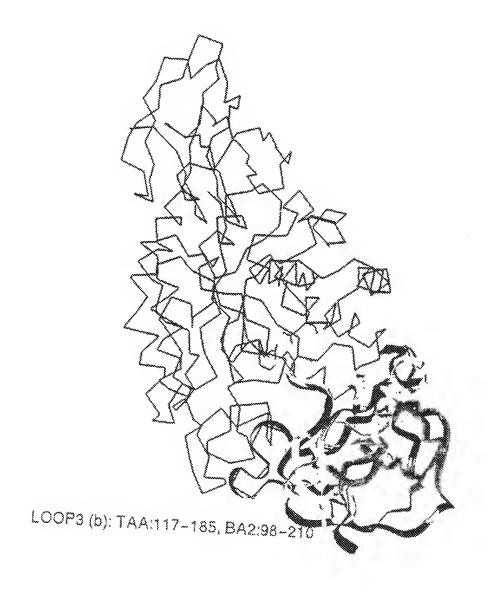


Fig. 3



Fig. 4

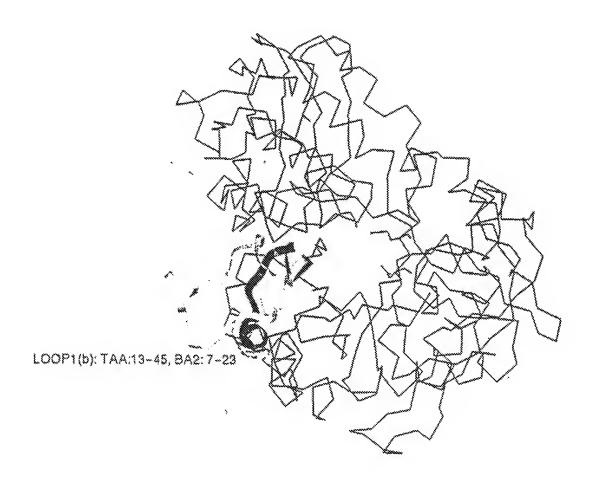


Fig. 5

SUBSTITUTE SHEET (RULE 26)



Fig. 6



Fig. 7

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CAT CAT AAT GGA ACA AAT GGT ACT ATG ATG CAA TAT TTC GAA TGG TAT TTG CCA AAT CAC инистистимочевычкеми 23 GOG ANT CAT TOG AND AND THE AGE GAT EAC CON GOT AND THA AND AGE ANA GOG ATA ACA άĭ GCT GTA TOG ATC COA CCT GCA TGG AAG GGG ACT TCC CAG AAT GAT GTA CGT TAT GGA GCC AVNIPPANKCTSONDVCYCA THE CAR THE THE GAT CIT GOA GAG THE AAC CAG AND GOG ACG OFF COT ACA AAA THE GGA Y D L Y D L C E F N Q R C T Y R T R Y G aca coc aac cag cta cag get geg gtg acc tet tta aaa aat aac gec att cag gta tat OUT GAT GTC GTC ATC AAT CAT AAA OUT GGA GCA GAT GGT ACG GAA ATT GTA AAT GCC GTA C D V V N N N N C C A D C T E I V N A V GAN OTG NAT COO AGO AND COA AND CAG GAN ACT TOA GON GAG TAT OUR ATA GAN OUG TOG E V N R S N R N Q E T S G E Y A I E A W ACS ASS TIT GAT TIT COT GGA AGS GGA AAT AAC CAT TOO AGO TIT SAG TGG CGG TGG TAT CHT THE GAT GOD ACA CAT TOG GAT CAG TOA CGC CAG CTT CAA AAC AAA ATA TAT AAA TTC в в о о тр м р о я х о ь о и х т ү х в AGO CGA ACA GGC AAG GCC TOG GAC TGG GAA GTT GAT ACA CAG AAT GGC AAG TAT GAC TAT R C T G R A W D N E V D T E N C N Y D Y CTT ATO TAT OCA CAC CTG GAT ATO GAT CAC CCA GAA GTA ATA CAT GAA CTT AGA AAC TGG LHYADVDHDHPEVIRELRHW 221 GGA GTG TGG TAT ACC AAT ACA CTG AAC CTT GAT GGA TTT AGA ATA GAT GCA GTG AAA CAT G V W Y T R T L R L D G F B L D A V K H ATA ANA THE ACC THE MCC AGA CAT TOO CTE ACA CAT CHO COT AND ACC ACA COT ANA CCA I K Y S F T R D W L T H V R N T T Z K P ANG THE GCA SIG SCE SAG TIT TOG AAA BAT GAC CEE GOT OCA ATT GAA AAC TAT TEE MAT R P A V A E F W K N D L G A I E N Y L N AND ACK AGT TOO ANT CAC TOO GIV TIT GAT GIT OUT CIC CAC TAT ANT THE TAC ART GOD K T S W N H S V F D V F L H Y S L Y S A

Fig. 8

301 TOT ART AGO GOT GOT TAT TAT GAT AGA AGA ART ATT TA ART GOT TOT GTG GAR ARA S N S G C Y Y D M R N I L N G S V V Q K . CAT CCA &CA CAT OCC OTT ACT TIT GIT GAT AAC CAT GAT TET CAG CCC OGG GAA GCA TIG H F T H A V T F V D N H D S O F G E A L GAA TOO TIT GIT CAA CAA TOO TIT AAA CCA CIT GCA TAT GCA TIG GIT CTG ACA AGG GAA CAA GOT TAT CCT TOO GTA TIT TAT GGG GAT TAC TAC GGT ATC CCA ACC CAT GGT GTT CCG O C A B Z A S A C D A A C I B J H C A B CCT ATC AAA TOT AAA ATA GAC COT CTT CTC CAG GCA CGT CAA ACT TIT GCC TAT GGT ACG A M K S K I D P L L O A R O T F A Y G T CAG CAT GAT TAC TIT GAT CAT CAT GAT ATT ATC GGT TOG ACA AGA GAG GGA AAT AGC TOC онругонноггонтаесне CAT CEA AAT TEA GOD CTT GOD ACC ATT ATC TEA GAT GOT COX COT GOT AAC AAA TOG ATG TAT OTO GGG AAA AAT AAA GCG GGA CAA GTT TOG AGA GAT ATY ACC GGA AAT AGG ACA GGC Y V G X N X Å G Q V N R D I T O N R T G ACC STC ACA ATT NAT GCN GAC GGA TOS SGT NAT TTC TCT GTT NAT GGN GGG TCG STT TCG T V T I N A D C W C N F S V N S C S V 9 481 GTT TOO GTG ANG CAA TAA v w v x q ·

Fig. 8 (cont.)

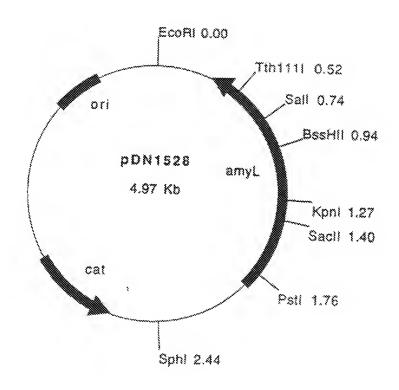


Fig. 9

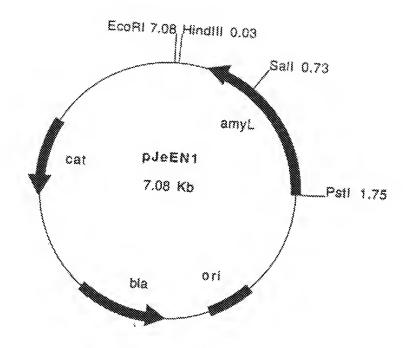


Fig. 10

International application No. PCT/DK 96/00057

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12N 9/28, C12N 15/56 According to international Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation ararched (classification system followed by classification symbols)

IPC6: Cl2N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE.DK.FI.NO classes as above

Electronic data have consulted during the international search (name of data base and, where practicable, search terms used)

WPI. CA. MEDLINE, BIOSIS

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No	
P,X	Dialog Information Services, File 5, BIOSIS PREVIEWS, Dialog accession no. 11619266, BIOSIS no. 98219266, Machius M et al: "Crystal structure of calcium-depleted Bacillus licheni- formis alpha-amylase at 2.2 A resolution", & Journal of Molecular Biology 246 (4), 1995, 545-559	1-17	

*	Dialog Information Services, file 155, MEDLINE, Dialog accession no. 08974640, MEDLINE accession no. 94289640, Svensson B: "Protein engineering in the alpha-amylase family: catalytic mechanism, substrate specificity, and stability", & Plant Mol Biol (NETHERLANDS) May 1994, 25 (2) p141-57	1-17	

X Further documents are listed in the continuation of Bax C.	X See patent family annex.
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- Special categories of osed documents.
- "A" document defining the general state of the art which is not considered to be of particular retevance
- "E" eriter document but published on or after the international filing date
- "It" document which may throw doubts on priority class(s) or which is ment to establish the publication date of another clusters or other special mason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other 899636U
- document published prior to the international filling date but later than the priority date claimed
- tater document published after the international filing date or primity date and not in conflict with the application but rated to understand the principle of theory underlying the invention
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- document of particular relevance: the claimed to vention cannot be considered to tavolve an inventive step when the document is cumbined with one or more other such documents, twen combination ne sat at lasticle correq a or moived gainst
- "&" document member of the same patent family

Date of the actual completion of the international search Date of mailing of the international gearch report n s -07- 1996 <u>5 July 1996</u> Name and mading address of the ISA. Authorized officer Swedish Patent Office Bex 5055, S-102 42 STOCKHOLM Yvonne Siösteen Facsimile No. + 46 8 666 02 86 Telephone No. +46 8 782 25 (8)

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The invention claimed relates to a method of constructing alpha-amylase variants with predetermined properties by comparing the three-dimensional structures of enzymes. The claims also include many alpha-amylase variants.

"A search for a special technical feature" as mentioned in PCT Rule 13.2 among the independent claims did not reveal a unifying, novel technical feature.

Accordingly, the following inventions were found:

- I Claims 1-17 focus on a method of constructing alphaamylase variants by comparing the tree-dimensional
 structure of a parent enzyme (Temamyl-like alpha-amylase)
 with another enzyme e.g. mammalie or fungal alphaamylases. The differences in structure are compared with
 the differences in function, whereafter new variants with
 new predictable characteristics are produced.
- II Claims 45-46 directed to a alpha-amylase variant that has decreased Ca2+ dependency,
- III Claim 47 directed to a alpha-amylase variant that exhibits higher activity below the ph-optimum than the parent enzyme.
- IV Claim 48 directed to a alpha-amylase variant having an increased thermostability and/or altered temperature optimum.
- V Claim 50 directed to a variant having reduced capability of cleaving an oligo-saccharide substrate close to its branching point.

Due to the complex construction of the claims and the fact that the search so far has not covered all aspects of the invention, it may be that further non-unity remarks can appear. If further searches are done, references might appear which will give furter a posteriori non-unity remarks.

Therefore, the search has been restricted to the first invention.

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Claims 18-43 are directed to a number of different variants that are composed of several inventions. They are, however, so complex and broad that no meaningful search can be done, especially as no special characteristic is linked to the groups of variants. It is for example unlikely that claim 18 concerns one invention. It is not believable that a change in any amino acid in one fragment for one/or none of the amino acids in a fragment of another enzyme gives an enzyme with the same new and valuable characteristic. The formulation of claims 18-43 is so complicated because of all the different combinations of amino acid substitutions.

Thus they do not comply with Art. 6. PCT prescribing that claims shall be clear and concise.

Form PCT/ISA/Dis (continuation of second sheet) (July 1992)

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	
X	Dialog Information Services, file 155, MEDLINE. Dialog accession no. 08958150, MEDLINE accession no. 94273150, Nakatani H et al: "Effect of modifying histidine residues on the action of Bacillus amylo- liquefaciens and barley-malt alpha-amylases", & Carbohydr Res (NETHERLANDS) Apr 16 1994, 257 (1) p 155-61	1-17	
¥		45-46	
*	J. MED. BIOL., Volume 229, 1993, C. Chang et al, "Crystallization and Preliminary X-ray Crystallographic Analysis of alpha-Amylase from Bacillus subtilis" page 235 - page 238	1-17	
A	WO 9100343 AZ (GIST-BROCADES N.V.),	X X X	
	10 January 1991 (10.01.91)	1-17	
Å	EP 0410498 A2 (GIST-BROCADES N.V.), 30 January 1991 (30.01.91)	1-17	
<i>A</i>	JOURNAL OF BACTERIOLOGY, Volume 166, No 2, May 1986, G. L. Gray et al. "Structural Genes Encoding the Thermophilic alpha-Amylases of Bacillus stearothermophilus and Bacillus licheniformis" page 635 - page 643	1-17	
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3 ×	WO 9535382 A2 (GISTBROCADES B.V.), 28 December 1995 (28.12.95), claims 1-2, abstract	45-46	
	WO 9418314 A1 (GENENCOR INTERNATIONAL), 18 August 1994 (18.08.94)	45-46	

International application No.
PCT/DK 96/00057

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C (Continu	ation). DOCUMENTS CONSIDERED TO BE RELEVANT		- 3/1/2-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1
Category*	Citation of document, with indication, where appropriate, of the relevant	passages	Relevant to claim N
Ą	Chemical Abstracts, Volume 108, No 11, 14 March 1988 (14.03.88), (Columbus, Ohio, US Buisson, G. et al, "Three dimensional structu porcine pancreatic alpha-amylase at 2.9 Å resolution. Role of calcium in structure and activity", page 325, THE ABSTRACT No 90927h, J. 1987, 6 (13), 3909-3916	re of	45-46
¥	Chemical Abstracts, Volume 112, No 15, 9 April 1990 (09.04.90), (Columbus, Ohio, USA Vihinen, Mauno et al, "Site-directed mutagene of a thermostable alpha-amylase from Bacillus stearothermophilus: putative role of three conserved residues", page 347, THE ABSTRACT No 135178r, J. Biochem 1990, 107 (2), 267-272	sis	45-46
A	US 4600693 A (KAREN L. KINDLE ET AL), 15 July 1980 (15.07.86)	5	45-46
A	Chemical Abstracts, Volume 112, No 19, 7 May 1990 (07.05.90), (Columbus, Ohio, USA), Holm, Liisa al, "Random mutagenesis used to probe the structure and function of Bacillus stearothermophilus alpha-amylase", page 351, THE ABSTRACT No 174785f, Protein Eng. 1990, 3 181-191	et.	45-46

International application No.

PCT/DK96/00057

\$	Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)
This inter	national search report bas not been established in respect of certain claims under Article 17(2)(a) for the following reaso
	Claims Ness.:
.000.00	because they relate to subject matter not required to be searched by this Authority, namely:
8	Daims Nos.: recause they relate to parts of the international application that do not comply with the prescribed requirements to suc n extent that no meaningful international search can be carried out, specifically:
	see next sheet
3. M c	izúrs Nos.;
.4 mm	ecause they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)
Box II O	bservations where unity of invention is lacking (Continuation of item 2 of first sheet)
Ibis Interni	sticael Searching Authority found multiple inventions in this international application, as follows:
	see next sheet
· 🔲 🔉	See next sheet all required additional search fees were timely paid by the applicant, this international search report covers all
	all required additional search fees were timely paid by the applicant, this international search repon covers all
	all required additional search fees were timely paid by the applicant, this international search report covers all all searchable claims. All searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment any additional fee. Only some of the required additional search fees were timely paid by the applicant, this international search reporters only those claims for which fees were paid, specifically claims him.
	all required additional search fees were timely paid by the applicant, this international search report covers all affacts that the search of the searched without effort justifying an additional fee, this Authority did not invite payment any additional fee.
· D Ass.	all required additional search fees were timely paid by the applicant, this international search report covers all acceptable claims. All searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment any additional fee. Only some of the required additional search fees were timely paid by the applicant, this international search reporters only those claims for which fees were paid, specifically claims Nos.: aims 1-17 directed to a method of constructing alpha-amylase variants of claims 45-46 directed to an alpha-amylase.
- D As - D As - C1 - sn	all required additional search fees were timely paid by the applicant, this international search report covers all archable claims. All searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment any additional fee. Only some of the required additional search fees were timely paid by the applicant, this international search report ers only those claims for which fees were paid, specifically claims Nos.
L D As an	all required additional search fees were timely paid by the applicant, this international search report covers all all searchable claims. all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment any additional fee. only some of the required additional search fees were timely paid by the applicant, this international search reporters only those claims for which fees were paid, specifically claims Nos.; aims 1-17 directed to a method of constructing alpha-amylase variants of claims 45-46 directed to an alpha-amylase. required additional search fees were timely paid by the applicant. Consequently, this international search report is required additional search fees were timely paid by the applicant. Consequently, this international search report is recuired to the invention first mentioned in the claims; it is covered by claims Nos.;
- D As - D As - C1 - sn	all required additional search fees were timely paid by the applicant, this international search report covers all affacts obtained. all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment any additional fee. only some of the required additional search fees were timely paid by the applicant, this international search report was only those claims for which fees were paid, specifically claims Nos.: aims 1-17 directed to a method of constructing alpha-amylase variants of claims 45-46 directed to an alpha-amylase. required additional search fees were timely paid by the applicant. Consequently, this international search report is required to the invention first mentioned in the claims; it is covered by claims Nos.:

information on patent family members

01/04/96

International application No. PCT/OK 96/00057

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0-A2-	9535382	28/12/95	MONE	**********		
0-AI-	9418314	18/08/94	NONE	men men men har har har har har har deb eger	*************************************	
S-A-	4600693	15/07/86	NONE	an an an an an der dei die die die	***************************************	